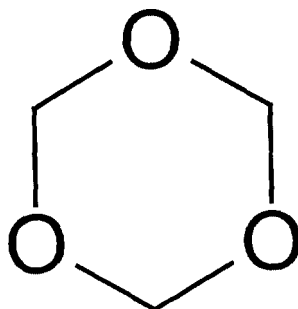


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1,3,5-TRIOXANE

CAS Number 110-88-3

USEPA HPV Challenge Program Submission

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Submitted by:

Trioxane Manufacturers Consortium
Members

BASF Performance Copolymers, LLC
(Formerly Ultraform Company)

and
Ticona

Prepared by:

Toxicology and Regulatory Affairs
One Rowlands Road
Flemington NJ 08822

908-806-7068

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Executive Overview

1,3,5-Trioxane (Trioxane) is a stable cyclic triether used primarily as a monomer for production of high-molecular weight polyacetals and secondarily as a chemical intermediate. Its production volume exceeds one million pounds per annum. It is estimated that over 99% of Trioxane production is used on the production sites as a monomer for making polyacetals. Polyacetals are strong and rigid resins that replace metals in many engineering applications. They are of little toxicological concern due to low bioavailability. FDA has approved polyacetals for food contact use and NSF has approved them for material in contact with potable water. Less than 0.2% of manufactured Trioxane is used in applications other than as a monomer. Exposure in these applications is limited by process controls, protective equipment and consumption in the application. Environmental releases are limited to vapor released to air at manufacturing sites.

The physical-chemical properties of Trioxane are well defined. It is a volatile solid at room temperature with a vapor pressure of 10 mm Hg. Trioxane is biodegradable in a waste water treatment facility but not considered readily biodegradable. Trioxane was found to have high hydrolytic stability in water at pH 4 to 9 with an estimated hydrolytic half-life at 25° C greater than one year. Vapors have an estimated photolytic half-life in air of approximately 25 hours, and predicted values for fugacity have been calculated with the MacKay model. Fish, daphnia and green algae are acutely affected by Trioxane only at concentrations greater than 2000 mg/l. Acute toxicity to mammals has been determined by oral, inhalation and dermal routes of exposure. Trioxane demonstrates a low order of toxicity with an oral LD₅₀ of about 8000 mg/kg in rats. Repeated-dose studies have been conducted by the oral or the inhalation route with some published studies using exposures up to twelve months duration. A NOEL of 200 mg/kg/day has been reported for a 28-day oral gavage exposure study in rats. Genotoxicity has been evaluated using multiple *in vitro* and *in vivo* experimental procedures covering both mutation and chromosomal aberration. The weight of evidence indicates a lack of significant genotoxic properties. Lack of significant reproductive toxicity can be predicted based on an ovarian function (estrous cycle duration) study, and two dominant lethal studies that determined testicular pathology in conjunction with fecundity and offspring parameters. Developmental toxicity results are available and, although fetotoxicity can be produced at high levels, this occurs only at maternally toxic doses. Trioxane, therefore, is not considered a specific developmental toxin. ADME studies indicate that Trioxane is readily absorbed from the gastrointestinal tract and is rapidly metabolized primarily to carbon dioxide. Although most of the ingested Trioxane is rapidly metabolized and excreted, some appears to become incorporated into tissue probably by way of the C-1 metabolic pool.

With regard to the HPV program, no additional testing is proposed for Trioxane as all required parameters are sufficiently well characterized by available data.

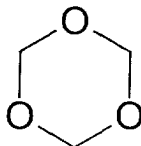
Testing Plan and Rationale

Testing Plan in Tabular Format

CAS Number 110-88-3 1,3-Trioxane								
	Information Available?	OECD Study ?	GLP Study?	Other Study ?	Estimation Method?	Acceptable?	Testing Recommended?	
HPV Endpoint								
Physical Chemical								
Melting Point	Y	N	N	N	N	Y	N	
Boiling Point	Y	N	N	N	N	Y	N	
Vapor Pressure	Y	N	N	Y	N	Y	N	
Partition Coefficient	Y	Y	N	Y	N	Y	N	
Water Solubility	Y	N	N	N	N	Y	N	
Environmental & Fate								
Photo-Degradation	Y	N	N	N	Y	Y	N	
Water Stability	Y	Y	Y	N	N	Y	N	
Transport	Y	N	N	N	Y	Y	N	
Biodegradation	Y	Y	N	Y	N	Y	N	
Ecotoxicity								
96-Hour Fish	Y	Y	N	Y	N	Y	N	
48-Hour Invertebrate	Y	Y	N	Y	N	Y	N	
72-Hour Algae	Y	Y	N	Y	N	Y	N	
Toxicity								
Acute	Y	N	N	Y	N	Y	N	
Repeated Dose	Y	Y	N	Y	N	Y	N	
Genetic Toxicology <i>in vitro</i>	Y	N	N	Y	N	Y	N	
Genetic Toxicology <i>in vivo</i>	Y	N	N	Y	N	Y	N	
Reproductive	Y	N	N	Y	N	Y	N	
Developmental	Y	Y	Y	Y	N	Y	N	

Introduction

1,3,5-Trioxane, CAS Number 110-88-3, (Trioxane) is a cyclic triether. It is a white stable solid with moderate water solubility used primarily as a monomer. Its structure is shown below:



The manufacturers estimate that over 99% of the production is used on the production sites as a monomer for making polyacetals. Polyacetals are very high molecular weight compounds of little toxicological concern. They are strong and rigid resins that can replace metals in many engineering applications. Polyacetals are **used** in a wide variety of applications, especially where low-friction properties are important. The polymer contains a very low level of free unpolymerized Trioxane and consumer exposure represents a negligible risk. FDA has approved polyacetal for food contact use and NSF has approved it for materials in contact with potable water. Less than 0.01 % of manufactured Trioxane is used as a co-solvent in a commercial (non consumer) application and less than 0.2% is used in any application other than as a monomer. Exposure in these applications is limited by process controls, protective equipment and consumption in the application.

A very small amount of the production had been used to produce fuel bars. As Trioxane is a flammable solid, material in small bar form is useful as a spot heat source. These bars were primarily used by the military for heating food when standard heating devices were unavailable. Currently, these bars are available through military surplus outlets and some companies selling camping and emergency supplies. A search of suppliers on the Internet revealed that all retailers of these fuel bars are selling military surplus materials. The manufactures believe that no new production is occurring or planned for this use. Fuel **bar use** was intended for the military and consumer use represents a potential uncontrolled exposure to a limited number **of** individuals and, as no new production of fuel bars is taking place, this potential exposure is self-limiting.

Although Trioxane is a volatile solid with a vapor pressure of 10 mm Hg at 20° C, little vapor exposure occurs. In the industrial setting, exposures are well controlled and air concentration is maintained at a low level in the workplace. As there are few sites of manufacture, the number of potentially exposed individuals in industry is small.

Numerous studies have been conducted on fate and toxicity of Trioxane. These studies are briefly reviewed in this rationale document describing how they meet the SIDS (Screening Information Data Set) end-points of the United States Environmental Protection Agency (USEPA) High Production Volume Challenge (HPV) program. Robust summaries have been prepared for key studies; supporting studies are referenced in these summaries.

Physical-chemical Data

Physical-chemical data for Trioxane are available from the literature and company sources.

Melting Point	64° C (1)
Boiling Point	114.5 ° C @ 759mm (1)
Vapor Pressure	10 mm @ 20° C (2)
Partition Coefficient	Log $K_{o/w}$ = -0.42 (3) Log $K_{o/w}$ = -0.47 (4)
Water Solubility	17.2 g/100 ml @ 18° C (1) 21.2 g/100 ml @ 25° C (1)

These properties indicate that Trioxane is a volatile solid with moderate water solubility. The value of the partition coefficient suggests that Trioxane will partition into water and has little potential for bioaccumulation.

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints.

Environmental Fate and Pathways

Photodegradation was estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The estimated rate constant is used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. An experimentally derived rate constant is listed in the APOWIN program. Using the default atmospheric hydroxyl radical concentration in APOWIN and the measured rate constant for reaction of Trioxane with hydroxyl radical, the estimated half-life of Trioxane vapor in air is approximately 25 hours (5).

Water stability has been determined for Trioxane using the Organization for Economic Cooperation and Development Guideline (OECD) 11 I protocol. In this study, Trioxane was found to be hydrolytically stable in the pH 4 to 9 range at elevated temperatures for six days, The estimated half-life for Trioxane in water at 25° C in this pH range is greater than one year (6).

Theoretical Distribution (Fugacity) of Trioxane in the environment was estimated using the MacKay model with standard defaults in EPIWIN v 3.05. (7) The results for distribution using a model calculated K_{oc} (adsorption coefficient based on organic carbon content) of 0.152 are:

○ Air	4.8 %
○ Water	53.4 %
○ Soil	41.6 %
○ Sediment	0.09 %

Trioxane was found not to be readily biodegradable showing little degradation in 28 days using a MITI Test (8). (Ready biodegradation tests use low levels of bacteria and simulate a lake or river; inherent biodegradation tests use high levels of bacteria and simulate a waste-treatment plant.) Volatilization was found to be a significant mechanism of loss under aeration conditions. A standard Zahn-Wellens (OECD 302B) inherent biodegradation test suggested biodegradation was occurring but the relative contribution of volatilization was not determined (9). A modified Zahn-Wellens test was conducted using a closed system with sodium nitrate as the oxygen source. In this study, greater than 90% of the DOC was removed in 18 days while only minimal loss of DOC was observed in controls without bacterial inoculum (10). This indicates that Trioxane is effectively removed from wastewater at a treatment plant.

Available data indicate that Trioxane will enter the environment by sublimation to a vapor and by means of wastewater. In wastewater treatment, volatilization and biodegradation are competing processes. Release to surface water is expected to result in volatilization within a period of days and no bioaccumulation. Photochemical oxidation will result in the ultimate degradation of Trioxane to water and carbon dioxide

Recommendation: No additional studies are recommended. The available data fill the HPV required endpoints.

Ecotoxicity

Trioxane was found to have minimal acute toxicity to typical aquatic species. The LC_{50} (96 - hour) for freshwater fish was found to be 4030 mg/l. (LC means lethal concentration; thus, an LC_{50} is the concentration resulting in death of half the fish in a particular time period, in this case, 96 hours.) The LC_0 and the NOEC (no observed effect concentration) were found to be 2 150 mg/l. The lowest concentration causing 100% mortality was 10000 mg/ml (11). This study closely followed the OECD 203 guideline, with the exception of the recommended species of fish. Supporting this study, a screening level study sponsored by Celanese in saltwater fish was conducted in which Sheepshead minnows (*Cyprinodon variegates*, 5 per group) were exposed to Trioxane at concentrations of 10000, 20000 and 30000 mg/l. In this study, the 96-hour LC_{50} was reported to be 16350 mg/l (12). Other support comes from modeling. The EPA ECOSAR model (a model developed by EPA to predict the aquatic toxicity of certain class of compounds) found in EPIWIN estimates the 96-hour LC_{50} for fish as 17000 mg/l (13).

Aquatic invertebrate toxicity was examined in two studies of *Daphnia magna*. In the first, which followed the OECD 202 guideline, the EC_{50} (4X-hour) and EC_0 (48-hour) were greater than 1000 mg/l (14). The second study was a screening level study, in which Trioxane was tested at 5000, 10000, 15000, 20000 and 30000 mg/l. In this study, the 48-hour EC_{50} was reported to be 15200 mg/l (12). Other support for a low-level of toxicity comes from modeling. The EPA ECOSAR model estimates the 48-hour LC_{50} for daphnia as 15000 mg/l (13).

Toxicity to aquatic plants was evaluated in two studies. The first, conducted by BASF Corporation, measured the growth rate of *Scenedesmus subspicatus* in the presence of Trioxane at concentrations up to 500 mg/l. Little inhibition was observed and the EC_{50} was found to be greater than 500 mg/l (15). This study is supported by an earlier screening-level study, sponsored by Celanese, in which Trioxane was tested for growth inhibition of *Selenastrum capricornutum*. In this study, algae growth was measured out to 14 days of exposure at levels of 1000, 5000 or 10000 mg/l. Significant inhibition occurred only at 10000 mg/l; the reported NOEC was 5000 mg/l (12).

All the reported ecotoxicity studies used nominal concentrations of Trioxane and not analytically measured concentrations. There are several potential sources of error in using nominal concentrations. The most important of these are mistakes in sample preparation, loss of material by volatilization from solution, hydrolysis of test material and biodegradation during the exposure period. Consistency of findings across laboratories indicates reliability in preparation of exposure levels. Volatilization was also considered an insignificant source of error based on the following two analyses. Volatilization can be estimated from the inherent biodegradation study where an aerated sample without bacterial inoculum was used as control.

Under these conditions, the air stripping of DOC (dissolved organic carbon) was 17% at 2 days and 27% at 4 days (10). An alternative means of estimation comes from EPIWIN which estimates the half-life of Trioxane in a river with a 1 meter water depth, a 1 meter per second current (well mixed) and a wind velocity of 5 meters per second to be 90 hours. Based on the EPIWIN estimate, excessive volatilization of Trioxane is not anticipated during a 2-day (daphnia) or a 4-day (fish) test. The worst-case condition, where the sample is well mixed and there is rapid exchange of air over the surface, indicates a loss of 50% over the 4 days of a fish test. As there was no mortality at the 2 150 mg/l level, Even with a 50% volatilization loss the LC_{50} still is in excess of 1000 mg/l.

Biodegradation can be eliminated as a means of significant test material loss since Trioxane is not readily biodegradable. Hydrolysis, likewise, has been shown not to be an important source of compound loss in this pH range. The concentration levels used in the fish tests and the supporting daphnia test (48-hour LC_{50} of 15,200 mg/l) were also far greater than the currently OECD acceptable maximum concentration limits of 100 mg/l for fish and 1 000 mg/l for daphnia. Thus, concentration errors, volatilization, biodegradation and hydrolysis can be eliminated as factors that would significantly change the conclusion that Trioxane has a low level of acute toxicity to typical aquatic organisms. It can be concluded that the ecotoxicity tests are adequate for the purposes of defining these SIDS endpoints. The ECOSAR model also supports the observed low toxicity to aquatic organisms.

Recommendation: No additional testing is required. The ecotoxicity results indicate that Trioxane is of low concern to aquatic environmental species. Although the studies were not conducted with measured concentrations of Trioxane, the established volatility and high initial levels provide adequate assurance of low acute toxicity.

Acute Toxicity

Oral Exposure

The acute oral toxicity was determined in male rats using water as vehicle. The high dose, 10000 mg/kg, produced mortality in four of five animals. No mortality was observed at 5000 mg/kg or lower doses. Target organs were not identified and the LD_{50} (dose resulting in 50% mortality) was calculated to be 8 190 mg/kg (16). This study is supported by a study in the literature that reports the acute oral LD_{50} to be 8500 mg/kg (17).

Inhalation Exposure

A well-documented guideline-like GLP study was conducted in 1986. The results appear reliable and show that the LC_{50} (inhalation concentration resulting in 50% mortality) for

Trioxane is greater than 10600 ppm for a 4-hour exposure. No target organs were identified. The high-dose was the maximum vapor concentration that could be achieved (18). This study is supported by a study in the literature that reports the acute inhalation LC_{50} to be greater than 6500 ppm ($>26000 \text{ mg/m}^3$) for a four-hour exposure (17).

Dermal Exposure

Trioxane produced no mortalities when administered by the dermal route to four albino rabbits at a dose of 3980 mg/kg body weight. In this study, the only signs of toxicity were a reduction in weight gain during the first week post-exposure and slight to moderate skin irritation noted at 24 hours (19).

Recommendation: No additional acute studies are required. Trioxane has been evaluated for acute toxicity by all three of the usual routes of exposure and the results demonstrate that Trioxane has low potential for producing acute toxicity.

Repeat Dose Toxicity

Oral Exposure

In a 28-day gavage study, five rats of each sex per group were exposed to 0, 40, 200, or 1000 mg/kg/day Trioxane in water vehicle. High dose animals of each sex showed a significant decrease in leucocytes and there was a tendency for a decrease in leukocytes at the 200 mg/kg/day level in males which did not reach statistical significance. In addition, high-dose males were found to have an increase in serum gamma-glutamyl-transpeptidase and high-dose females were found to have increases in serum gamma-glutamyl-transpeptidase. High-dose females also displayed a decrease in serum protein and glucose. The 200 mg/kg/day level was considered a NOAEL. Adverse effects on the testes were reported in 1/5 high-dose males (20).

Inhalation Exposure

In a 1983 14-day study, rats were exposed to Trioxane at concentrations of 0, 103, 984 or 4940 ppm. Rats exposed to 4940 ppm Trioxane vapor displayed signs of toxicity including decreases in weight gain, righting reflex and grip strength. A dose-related increase in the incidence of splenic atrophy, decreases in spleen weights, and squamous metaplasia of the anterior nasal passage were observed at 984 and 4940 ppm. Animals exposed to 103 ppm showed a low incidence of rhinitis indicative of minor irritation and males of this group were found to have reduced spleen weights as compared to controls. The LOAEL (lowest observed adverse effect concentration) for males was considered to be 103 ppm, this level was considered a NOAEL for females (21).

There is good correlation between the oral and inhalation studies in terms of dose and target organ response. For example, both studies showed reduction in WBC's. In the oral study, this reduction was significant at 1000 mg/kg/day and for the inhalation dosing it was significant at 4950 ppm (equivalent to 4950 mg/kg/day total inhaled dose assuming a 270 ml minute volume for a 350 gram rat (22)). Splenic weight changes occurred at an oral dose of 1000 mg/kg/day and at inhalation doses of 984 ppm for females and 103 ppm for males (the 103 ppm dose was a NOAEL for all other parameters in the inhalation study). The NOAELs and LOAELs are compared in the table below.

Sex	Parameter	28-Day Oral (mg/kg/day)	14-Day Inhalation (mg/kg/day)*
Male	NOAEL	200	<105
	LOAEL	1000	105
Female	NOAEL	200	105
	LOAEL	1000	1005
* Assuming a 270 ml minute volume for a 350-gram rat, and 100% absorption, not correcting for days/week dosed or study duration.			

From an examination of this table it appears that rats are slightly more sensitive to the adverse effects of Trioxane by inhalation than by gavage. If one considers that the inhalation absorption is probably less than 100% and that the inhalation study duration was about half that of the gavage study, inhalation appears to be the more sensitive route. It is speculated that after oral administration, first-pass metabolism by the liver detoxifies a portion of the Trioxane reducing the oral toxicity compared to inhalation. This possibility is supported by the metabolism data that indicate Trioxane is rapidly metabolized and primarily excreted as carbon dioxide from the lungs (see Metabolism and Special Studies).

Recommendation: No additional testing is required. The SIDS repeated-dose endpoint is filled by two studies of good reliability that cover the two important routes of exposure. Additional supporting data from longer-term oral and inhalation studies are also available (23, 24, see robust summaries of the repeated-dose studies for summary details of the longer term studies).

Genetic Toxicity

The SIDS/HPV requirement for genetic toxicity screening is for two end-points: generally one sensitive to point mutation and one sensitive to chromosomal aberrations. In the case of Trioxane, these requirements are fulfilled by multiple studies covering both types of end-points.

Genetic Toxicology in vitro

A *S. typhimurium* reverse mutation assay (BASF, 1988; robust summary attached) was conducted in four strains of bacteria using a triple plate, independent repeat design. It is supported by a single-plate test from another laboratory using five strains of bacteria (TA98, TA100, TA1535, TA 1537 and TA1538) exposed with and without S-9 activation at eight concentrations of Trioxane from 0.5 to 5000 micrograms per plate. No mutagenic response was recorded in this study (25). These studies show that the material is not genotoxic to *S. typhimurium* under these conditions.

Two additional studies in prokaryotes produced negative results. In a triple plate test, with preincubation in the presence and absence of S-9 with strains TA97, TA98, TA100 and TA1535 at concentrations from 100 to 10000 micrograms per plate, Trioxane was reported to be without mutagenic activity (26). The mutagenic activity of Trioxane was investigated in five strains of *S. typhimurium*: TA1535, TA1537, TA1538, TA98 and TA100 with and without activation by liver microsomes (induced with Aroclor 1254). It displayed no mutagenic activity under any of these conditions (27).

A mouse lymphoma assay showed no genotoxic activity in the absence of metabolic activation; however, genotoxicity was indicated in the presence of metabolic activation. The dose-dependent increase in the number of mutants under activation conditions was associated with significant cytotoxicity at the higher dose levels (28).

A cell transformation study was conducted using C3H10T-1/2 cells. A wide range of concentrations was tested using both 1 I -day and 38-day incubations following a 24-hour exposure to Trioxane. No increase in the number of transformed colonies or transformed foci was noted at any concentration of test material (29).

Genetic Toxicology in vivo

A published mouse micronucleus study showed no genotoxic activity in BALB/c mice. Mice were given Trioxane by intraperitoneal injection in two doses at 24-hr intervals. Intraperitoneal administration of Trioxane produced no chromosomal damage resulting in erythrocyte micronucleus formation, even at highly toxic doses (30).

Trioxane was tested for its ability to induce DNA repair (Unscheduled DNA synthesis; UDS) in *vivo* using rat hepatocytes. Trioxane, dissolved in water, was administered once orally to male Wistar rats at dose levels of 250 mg/kg, 500 mg/kg, 1000 mg/kg or 2000 mg/kg body weight. Treatment with test substance did not lead to an increase in the mean number of net nuclear grain counts from 500 mg/kg up to 2000 mg/kg body weight at any exposure time in rat hepatocytes. The lowest dose group (250 mg/kg body weight) was not evaluated since the two higher dose groups were negative. Under these test conditions, Trioxane was considered negative in the rat hepatocyte in *vivo* UDS assay. (3 1)

Two dominant lethal studies have been conducted in rats. An oral study was conducted in which male rats received Trioxane daily for eight weeks and effects on fertility were evaluated for in a weekly mating design. Oral doses of 0, 850 and 1700 mg/kg/day had no effect on male fertility indexes. Pregnancy outcomes were also comparable to controls. No dominant lethal effect was observed under these conditions (33).

The second dominant lethal study was an inhalation study in which male rats were exposed to Trioxane 5 hours daily, 5 days a week, for 12 months, at concentrations of 2500 mg/m³. At the end of the 12-month exposure period, males were mated with females in a ratio of 1:2 through one week. Dams were sacrificed 13-14 days after the middle of mating intervals. No increase in the number of preimplantation losses, dead implants and live fetuses per female was noted in any treated group as compared to an appropriate control group. Trioxane did not affect the fertility of males. The study did not reveal induced dominant lethal mutations in germ cells from inhalation exposure to Trioxane (33).

Recommendation: No additional testing is required. The SIDS requirement for genetic testing has been fully met by a battery of several studies both in *vitro* and *in vivo* and sensitive to both mutations and chromosome aberrations. With the exception of the mouse lymphoma in the presence of metabolic activation, all studies demonstrated lack of genotoxic activity. The weight of evidence suggests low genotoxic potential for Trioxane.

Reproductive Toxicity

Trioxane has not been tested for reproductive toxicity using a standard reproductive testing protocol; however, several studies are available which supply the information necessary to assess potential reproductive effects. The SIDS Manual (32) gives guidance for the amount of information necessary to fill the toxicity to reproduction requirement in the absence of OECD guideline (OECD 415, 416, 421 or 422) studies. The SIDS manual indicates that the reproductive endpoint can be met alternatively by stating “*when a 90-day repeated-dose toxicity study is available and demonstrates no effect on reproductive organs, in particular the testes,*

then a developmental study (e.g. OECD Test Guidelines 414) can be considered adequate to complete information of reproduction/developmental effect." In the case of Trioxane, comprehensive and reliable long-term repeated dose studies are not available; however, testicular and ovarian functions were evaluated using other protocols and a reliable developmental toxicity study is available.

Testicular Function Tests

In a dominant-lethal study, male rats that received Trioxane daily for eight weeks were evaluated for effects on fertility in a weekly mating design. Oral doses of 0, 850 and 1700 mg/kg/day Trioxane (up to 20% of the LD₅₀) had no effect on male fertility indexes. Pregnancy outcomes were also comparable to controls. Although no functional deficit in fertility was recognized in these males, microscopic evidence of testicular degeneration and alterations in spermatogenesis were noted (33). Focal necrosis of the seminiferous epithelium was reported in 1/10 control, 3/10 850-mg/kg males and an unspecified number of high dose males. It was reported that the testicular lesions were bilateral in 3/10 high-dose males. Severity was reported as being dose dependent.

In a 12-month inhalation study (inhalation dominant-lethal study, robust summary attached), male rats were exposed to Trioxane 5 hours daily, 5 days a week, for 12 months, at concentrations of 2500 mg/m³ (equivalent to 580 mg/kg/day assuming a 270 ml/min minute volume, a 350-gram rat and 100% absorption (22)). At the end of the 12-month exposure period, males were mated through one week with female rats in ratio 1:2. The autopsy of dams was carried out 13-14 days after the middle of mating intervals. No increase in the number of preimplantation losses, dead implants and live fetuses per female was noted in any treated group as compared to an appropriate control group. Trioxane did not affect the fertility of males, and no histopathological changes in the testes were reported. (It cannot be definitively determined that testes were examined macroscopically or microscopically; however, in the oral dominant-lethal included in the same publication, testicular effects by histopathologic examination were noted. Thus, it is anticipated that the testes were examined). The study did not reveal induced dominant lethal mutations in germ cells from inhalation exposure to Trioxane (33).

Ovarian Function Test

Starek and Barazski (34) studied the effect of Trioxane exposure on the estrous cycle of rats. In this study an aqueous solution of Trioxane was administered by gavage to female rats, 5 days per week for 7 weeks, at doses of 190, 580 or 1160 mg/kg/day. They reported that a significant increase in the mean duration of the estrous cycle, mainly due to lengthening of the diestrus, was only noted in the 6th and 7th week of compound administration at a dose of 1160 mg/kg. Three weeks after cessation of treatment, the cycle had returned to normal in the affected group. A statistically significant dose-related decrease in body weight gain was observed in females

given test material at all three treatment levels and Trioxane-induced behavioral changes were seen in the high-dose group. It was, therefore, concluded that exposure to Trioxane did not affect the sexual cycle unless other overt signs of toxicity were present. Histopathology or other measures of toxicity were not recorded and it is assumed they were not collected; however, estrous cycle activity is itself a strong indication that Trioxane does not interfere with ovarian function.

Results from this study correspond well with the 28-day gavage study (20) where a NOAEL of 200 mg/kg and a LOAEL of 1000 mg/kg were reported. In the ovarian function test, the 580 mg/kg dose group would have been the NOAEL (based on body weight) had the study been terminated at 28-days. It was only after six weeks of dosing that the body weights became significantly different for the 580 mg/kg group and only at the end of eight weeks dosing for the 190 mg/kg group.

The logical value of the estrous cycle assay as a sensitive indicator of female reproductive toxicity is high since it requires both ovarian and neuro-hormonal integrity. It is also supported by published literature. May and Finch (35) advocated estrous cycle as a method to test presumptive reproductive toxins noting that quantitative changes in cycle length are preferred. They stated that cycle length distributions indicate more subtle impairments in reproductive function than other available methods; however, they require more refined data analysis. Chapin et al. (36), reported on the relationship among reproductive endpoints in Swiss mice using the reproductive assessment by continuous breeding database, containing data for 72 chemicals at that time. In these studies, it was noted that longer estrous cycle time correlated well with reduced pup numbers. They concluded that estrous cycle length is a useful surrogate of overall reproductive function for females. The EPA has recently added estrous cycle length determinations to the multi-generation testing guidelines and noted, "The new endpoints for monitoring pubertal development, semen quality, and estrous cyclicity will better enable determination of the affected sex, target organ, and life stage following exposure throughout the life cycle." (37).

Recommendation: No additional testing is required. The available data show effects on female reproductive function only at systemically toxic doses, and no functional deficits in male reproductive function after long-term dosing. Additional testing would not add significantly to the information necessary to define the reproductive toxicity for this material.

Developmental Toxicity

A developmental toxicity study for Trioxane was completed by Hoechst Marion Roussel (HMR) in 1998 using the OECD 414 guideline and also in accord with the appropriate EU and US EPA guidelines (38). In this gavage study, groups of pregnant rats were treated at 0, 100, 315 or 1000 mg/kg/day from days 7 to 20 of pregnancy. Results of this study indicate that Trioxane is not a specific developmental toxin. The developmental NOEL was found to be 100 mg/kg/day while a maternal NOEL was not defined since corrected dam body weight gain was affected in all treated groups. The higher dose levels, which were maternally toxic, produced numerous variations in the offspring indicative of fetotoxicity associated with the maternal toxicity. Two developmental toxicity studies of Trioxane have been published and these are summarized in the robust summary for the developmental toxicity study (39,40).

Recommendation: No additional testing is required. The HMR study meets the HPV requirements for detailed and well-documented developmental toxicity data and the results are generally supported by two published studies. When the data are examined as a whole, Trioxane, when dosed using the daily regime recommended in the OECD guidelines, only causes adverse developmental effects in the presence of maternal toxicity.

Metabolism and Special Studies

A study was conducted to investigate Trioxane distribution, excretion and metabolism. Male Wistar albino rats were administered a single dose of ^{14}C Trioxane at either 40 mg/kg or 400 mg/kg. Exhaled air was found to be the main route of ^{14}C elimination, which was mainly in the form of CO_2 . Elimination of Trioxane by exhalation during the first 12 hr following the administration of 40 mg/kg was rapid with a half-life of 3.5 h. At 400 mg/kg, Trioxane was eliminated 77% as CO_2 and 8% as unchanged Trioxane in exhaled air. Also in this 12-hour period, about 3% of the ^{14}C was excreted in the urine in the form of unchanged Trioxane. This demonstrates that Trioxane is rapidly and extensively metabolized. A small portion of the administered Trioxane is removed more slowly and may become a part of the C-I metabolism pool. Trioxane elimination from the plasma, at a dose of 40 mg/kg, showed biphasic elimination, with half-lives of 4.5 and 72 hours. The primary elimination route appears to be metabolism and excretion of carbon dioxide from the lungs. Distribution studies indicated that liver had the highest ^{14}C concentration of examined organs while fat tissue and brain had the lowest. The authors concluded that Trioxane is rapidly eliminated and is not expected to accumulate within tissue (41).

Another metabolism study of Trioxane was conducted by administering 2500 mg/kg ^{14}C -Trioxane to three Sprague-Dawley rats of each sex weighing between 221 and 246 grams. The radioactive Trioxane was administered by oral gavage as a water solution. Exhaled carbon

dioxide, urine and feces were collected for the intervals 0-12, 12-24, 24-48 and 48-72 hours. Radioactivity was determined in each matrix for each time interval. Overall, during the 72 hours after administration, ^{14}C elimination was about 72% in exhaled air as CO_2 . About 15% was recovered in urine and less than 1% in feces. About 2% of the initial dose remained in tissues after 72 hours with the majority being found in the liver. Total ^{14}C recovery was about 90%. Mean data for radioactivity trapped in sodium hydroxide (presumed to be carbon dioxide) at each time interval for each group is given below.

	Percent of Total Radioactivity Exhaled as Carbon Dioxide	
Time Interval	Males (n=3)	Females (n=3)
0-12	23.3	16.1
12-24	26.7	25.0
24-48	9.1	11.6
48-72	0.7	0.8
Total	73.8	71.5

It was concluded that Trioxane is readily absorbed from the gastrointestinal tract and rapidly metabolized to CO_2 ; no sex difference was found in metabolism by these measures (42). Although a half-life was not calculated in this study, it is apparent from the data that most of the radioactive carbon dioxide was exhaled in the first 24 hours. More radioactive carbon dioxide was exhaled in the second twelve-hour period than the first. It can be speculated, based on the metabolic results at lower doses that this probably represents saturation of the metabolic pathways at this 2500 mg/kg dose. The lower carbon dioxide exhalation in the first twelve hours may be accounted for by a time lag between administration and absorption.

Studies were performed in an attempt to determine if Trioxane reacts hydrolytically with whole blood to form simpler species. Pooled control rat blood was incubated with Trioxane at 0, 10, 100 or 500 mg/l for one hour at 37° C. Recovery of unchanged Trioxane after the one-hour incubation was used as a measure of possible metabolism. The average recoveries of Trioxane were 73, 93 and 80%, respectively, for 10, 100 and 500 mg/l fortifications of a homogeneous sample of pooled control rat blood. The investigators concluded these results show that the majority of Trioxane incubated with whole blood for one hour could be recovered. This suggests that little, if any hydrolysis or metabolism occurs with blood alone (43).

A study in pregnant rats was conducted to determine tissue distribution and binding of ^{14}C activity at various time intervals following oral administration of radiolabeled Trioxane. A single oral dose of universally labeled ^{14}C -Trioxane at 40 mg/kg was administered to pregnant rats. Animals were killed on the 21st day of gestation 3, 24, or 48 hours after administration of Trioxane. In maternal rats, 3 hours after administration, the highest levels of total radioactivity

were found in liver and plasma, followed by a gradual decline with time. The level of ^{14}C -activity in the fetus was comparable to that of the maternal kidney throughout the study. The radioactivity in the fetal kidney and liver 48 hours after administration was higher than at 3 hours after administration. A slow decline in radioactivity was observed with time in the fetal brain, skin and carcass. After 48 hours, however, radioactivity in the fetal kidney and brain was more than twice as high as in the corresponding maternal organs. Three hours following ^{14}C -Trioxane administration, 35 and 41 % of total radioactivity in maternal liver and kidney, respectively, was firmly bound to macromolecules, while the fetal liver and kidney showed 100 and 72% binding of radioactivity, respectively (44). The relative accumulation of radioactivity in fetal tissue is expected if Trioxane is metabolized and enters the C-1 pool, perhaps by way of tetrahydrofolate. The fetal tissues are undergoing rapid growth and incorporation of C-1 moieties as compared to maternal tissues. It could not be distinguished if Trioxane was binding to fetal tissues by means of a reactive metabolite or by orderly incorporation by way of the C-1 pool. It was established, however, that Trioxane is rapidly metabolized to carbon dioxide and C-1 pathways would be expected to saturate with radioactive carbon from Trioxane. Thus, C-1 pathways are considered likely to be responsible for much of the fetal incorporation of radioactive carbon.

These ADME studies conducted indicate that Trioxane is readily absorbed from the gastrointestinal tract and is metabolized primarily to carbon dioxide. Either it or a metabolite crosses the placenta and is incorporated into fetal tissues. Although most of ingested Trioxane is rapidly metabolized and excreted, some appears to become incorporated in tissue. With the available data, it cannot be distinguished definitively if a metabolite of Trioxane reacts with tissue macromolecules directly or if it enters the metabolic C-1 pool where it is incorporated into tissue, or both. Data from other metabolism studies implicate the C-1 pool mechanism as important in the incorporation of radioactive carbon from Trioxane in tissues.

Conclusions

With regard to the parameters specified in the EPA HPV Challenge program, the available data fill all of the requirements for chemical parameters, fate and toxicity information. No additional studies are proposed for this program.

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